CHAPTER I: INTRODUCTION

1.1. Background

Opisthorchiasis is one of the food-borne neglected tropical diseases with poor prognosis and high fatality rate. The disease is caused by the infection of one of the human liver fluke species, *Opisthorchis viverrini* (*Ov*), where it creates a significant disease burden, particularly in the Southeast Asia region (León et al., 2018). Thailand has been one of the countries in Southeast Asia with a high prevalence of Opisthorchiasis. The initial report of *Ov* infection in the 1950s conducted in Thailand showed that certain villages of northeast Thailand has prevalence nearly 100%. Even after 30 years later, the prevalence of *Ov* infection was unable to be suppressed in the Chonnabot district of Khon Kaen Province, thus confirming that Khon Kaen is one of the opisthorchiasis hot spots in the Northeast Thailand (Sithithaworn et al., 2012). Another study confirming the infection prevalence in 2008, reported by León et al. (2018), is shown in figure 1. The research was focusing on six village clusters that are located surrounding the Lawa Lake, including Lawa, Khok Samran, Don Po Daeng, Ban That, Nong Na Kwan, and Chi Kok Kho. Four out of six villages have infection prevalence more than 50% where the highest is located at the southernmost to the lake (Nong Na Kwan) at 70%.

The epidemiology agent of opisthorchiasis is transmitted to humans via ingestion when people consume the raw fish containing the infective stage metacercaria. The parasite will be matured inside the bile duct as they attach to the mucosal membrane of cholangiocytes and mature. The presence of the fluke inside the bile duct therefore induces inflammation and causes hepatobiliary abnormalities, including cholangitis, obstructive jaundice, hepatomegaly, periductal fibrosis, cholelithiasis and bile duct cancer or cholangiocarcinoma (CCA) (Sripa et al., 2007). The ability of this parasite in causing chronic and persistent infection associated with carcinogenesis are caused by several factors, including the parasite excretory/secretory (ES) products.

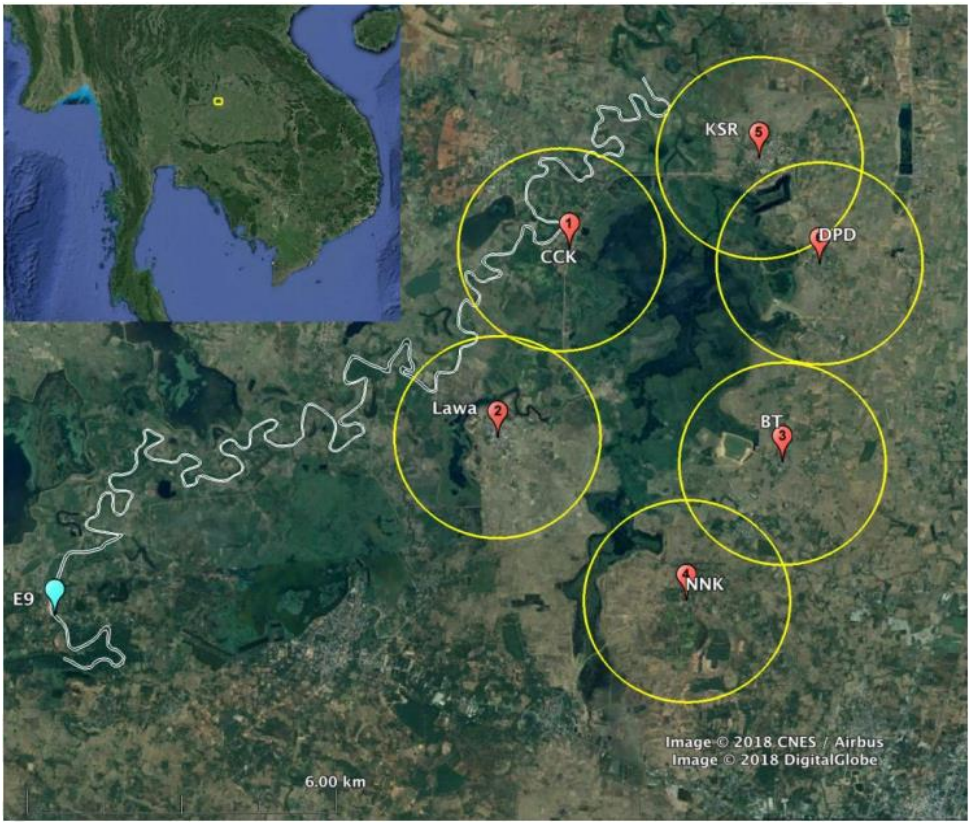

Figure 1. Prevalence of opisthorchiasis in six villages surround the Lawa Lake in 2008. KSR: Khok Samran; DPD: Don Po Daeng; BT: Ban That; NNK: Nong Na Kwan; Lawa; CCK: Chi Kok Kho. Reprinted from 'Modeling Liver Fluke Transmission in Northeast Thailand: Impacts of Development, Hydrology, and Control,' by León et al., 2018, Acta Tropica.

The ES products contain several important uncharacterized proteins that hypothetically play important roles in pathogenesis. Some of the proteins that have been successfully identified and functionally characterized are including growth factor granulin, thioredoxin as an antiapoptotic enzyme, thioredoxin peroxidase that acts as antioxidants, and some other proteases (Smout, M. J. et al., 2009; Matchimakul et al., 2015; Suttiprapa, S. et al., 2008). One of the most recent findings of the *Ov*-ES products is the N-glycosylated M60-like metallopeptidase (MP), with the most abundant type: *Ov*-M60-like 1 MP (Ta et al., 2019).

The recombinant *Ov*-M60-like 1 MP (r*Ov*-M60-like 1 MP) has been proven to have a mucinase activity that tested against bovine submaxillary mucin (Ta et al., 2019). Initially, proteomics and bioinformatics analysis identified that these proteins contain an N-terminal carbohydrate-binding domain and a C-terminal M60-like domain (Suttiprapa, unpublished). These metallopeptidases have

zincin metallopeptidase HEXXH motif (Suttiprapa, unpublished data). Many proteins with this motif have been found in the secreted proteins from several known pathogens and animal host mucosa-associated microorganisms, ranging from mutualists to pathogens (Cerdà-Costa, N., & Gomis-Rüth, F. X., 2014; Nakjang et al., 2012). Those revealed characteristics of M60-like MP might imply in the successful colonization of *Ov* in the bile duct mucosal surface. Mucins inside the mucosal membrane are glycoproteins that provide a physical barrier from pathogens and many foreign materials (Linden et al., 2008). These mucin productions and functions are known to be deregulated as inflammation and cancer occur (Tarang et al., 2012). Also, mucinase enzymes produced by many pathogens are proven to have the ability to degrade mucins (Rousseau and Swallow, 2012). In the study conducted by Ta et al. (2019), it was shown that the r*Ov*M60-like 1 MP could disrupt the mucous layer which may expose the bile duct epithelium to toxic substances leading to chronic tissue damage and repair. These confirm that the M60-like 1 MP plays a vital role in the CCA formation.

Apart from the unclear CCA formation in the opisthorchiasis, the currently available diagnostic methods for opisthorchiasis, which lay on microscopic reading, are still low in sensitivity and specificity. Hence early detection for the acute infection is challenging resulting in the incomplete measure and higher fatality rate. Even though opisthorchiasis induces and causes antibody responses, the current and frequently used technique to diagnose *Ov* infection is the formalin ethyl-acetate concentration technique (FECT), Polymerase Chain Reaction (PCR), and immunological techniques such as Enzyme-Linked Immunosorbent Assay (ELISA) or western blot which are found requiring a well-trained personnel and laboratory with sufficient capacity building (Worasith, C. et al., 2015). Hence, it will be advantageous if serodiagnosis could be an alternative approach as it is faster, more sensitive, and less time-consuming.

Previously, somatic and whole ES proteins have been used as diagnostic antigens in the laboratory but resulted in several problems. Using fluke-derived antigens result in an unstable and inconsistent quality of the products. The production was also tedious and low in reproducibility. Also, cross-contamination with other trematodes antigens frequently occurred. Meanwhile, a recombinant

antigen has the potential to be produced on a large scale, faster, and more promising than somatic and ES products. Since the *Ov*-M60-like 1 MP is the most abundant protein in the *Ov*-ES products with mucinase activity, we hypothesized that the recombinant *Ov*-M60-like 1 MP could be a promising serodiagnostic marker for opisthorchiasis.

This study is aimed to assess the potential of *Ov*-M60-like 1 MP as a diagnostic marker for opisthorchiasis. Proteomics approaches such as recombinant protein technology, western blot, and sandwich ELISA were utilized to produce the recombinant M60-like 1 MP and to test whether the recombinant antigen can be bound to the M60-like 1 antigen inside infected host serum. In addition, immunolocalization was conducted to observe the location of the M60-like 1 metallopeptidase inside the fluke, that enhance our understanding about the fluke and pave the way for further studies on host-parasite interaction during opisthorchiasis.

1.2. Research Question

Could *Ov*-M60-like 1 metallopeptidase be a serodiagnostic marker for opisthorchiasis in humans?

1.3. Hypothesis

H0: *Ov*-M60-like 1 metallopeptidase could not be used as serodiagnostic marker for opisthorchiasis.

H1: *Ov*-M60-like 1 metallopeptidase could be used as a serodiagnostic marker for opisthorchiasis.

1.4. Objectives

The objective of this research was to assess the capability of *Ov*-M60-like 1 metallopeptidase as a serodiagnostic marker by its possibility to be detected in infected host serum.